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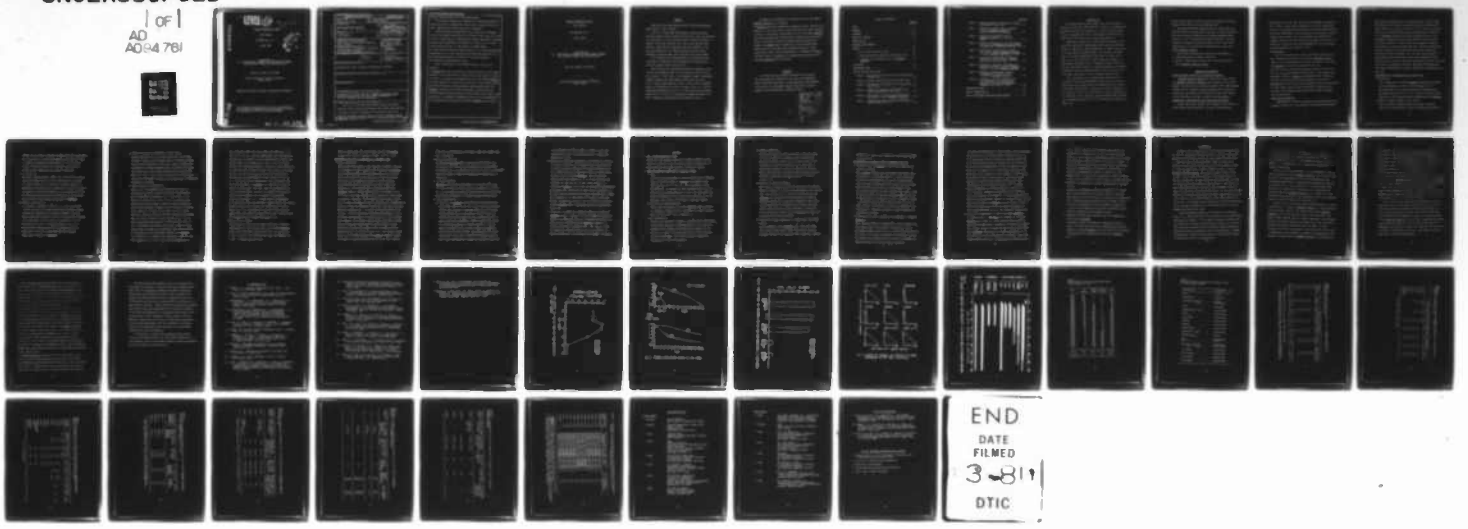

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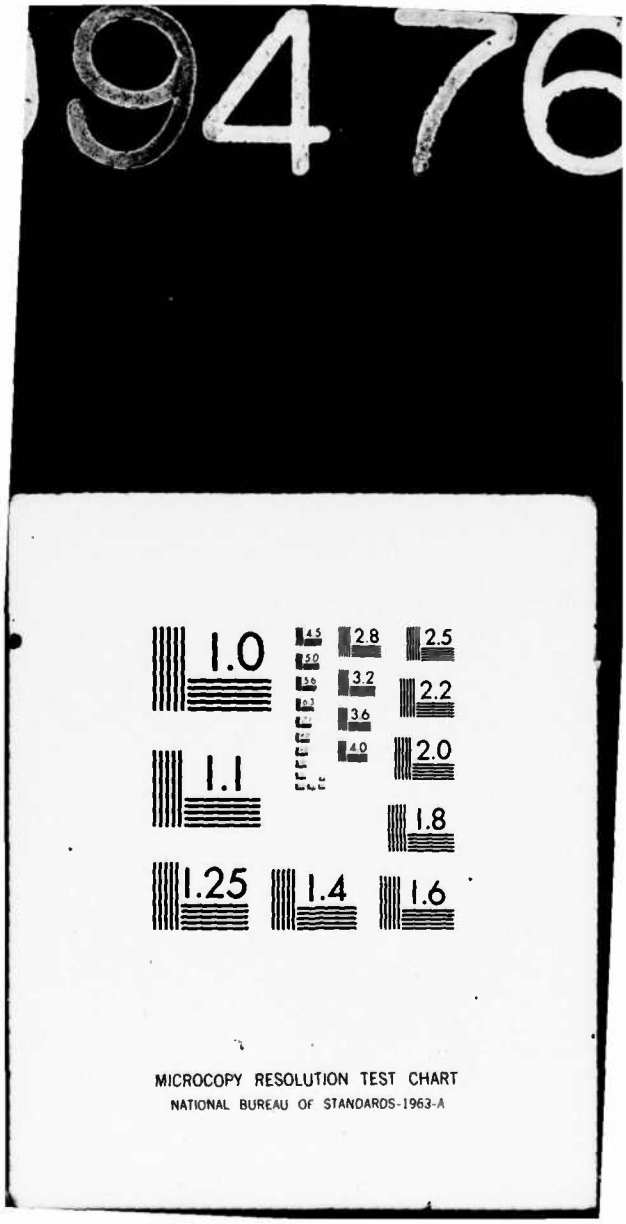
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KOREAN HEMORRHAGIC FEVER

Final Report

HO WANG LEE, M. D.

March 1980

Supported by
U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Grant No. DAMD 17-79-G-9455

Korea University College of Medicine
Seoul, Korea

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Lee et al. discovered the causative agent and animal reservoir host of Korean hemorrhagic fever (KHF) and perfected a serologic test for diagnosis of the disease. Lee et al. reported the agent causing similar clinical syndrome through

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Eurasia share antigens with the Korean agent.

This report presents the results on (1) characterization of KHF virus and (2) transmission of the virus in animal reservoir host, employing the immunofluorescent technique.

Physico-chemical properties of the virus are as follows: (a) The virus passed through 100 nm millipore filter but not 50 nm filter. (b) RNA virus. (c) Deoxycholate, ether, chloroform, acetone, benzene and fluorocarbon inactivated the virus. (d) U.V. light inactivated the virus. (e) The virus is lyophilizable. (f) Infectivity of virus is stable at pH 7.0 - 9.0 but inactivated completely at pH 5.0 (g) The virus was relatively stable at 4° C but inactivated readily at above 37° C and (h) Oridinary disinfectants inactivated the virus.

Virus transmission in the animal reservoir host are as follows:

a) Apodemus mice were infected by intranasal route. b) The large amounts of virus was found in saliva and urine but small amounts in feces. c) Horizontal transmission of the virus among Apodemus occurred beginning 10 days after inoculation of the source cohort, and further groups of animals became infected when caged with Apodemus infected up to 38 days previously. Results were not different when ectoparasitized and clean animals were used in the experiment. d) Attempts to demonstrate the virus in suspensions of ectoparasites harvested from infected Apodemus were failed.

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SUMMARY

There were 364 hospitalized cases of Korean hemorrhagic fever (KHF) in 1979 in Korea.

Lee et al. discovered the causative agent and animal reservoir host of KHF and perfected serologic test for diagnosis of the disease in 1978. Etiologic agent of KHF was named as Hantaan virus after Hantaan river that runs along the 38th Parallel. Employing the immunofluorescent technique it has been determined that the agents causing similar clinical syndromes through Eurasia share antigens with Korean agent.

This technique also made possible to characterize the virus and an examination of the dynamics of transmission of the virus among animal reservoir host.

The following are some characteristics of Hantaan virus. 1) The virus passed through 100 nm millipore filter but not 50 nm filter. 2) RNA virus. 3) Deoxychlate, ether, chloroform, acetone, benzene and fluorocarbon inactivated the virus. 4) U.V. light inactivated the virus. 5) The virus is lyophilizable. 6) Infectivity of virus is stable at pH 7.0 - 9.0 but inactivated completely at pH 5.0. 7) The virus was relatively stable at 4° C - 20° C but inactivated rapidly at above 37° C and 8) Ordinary disinfectants inactivated the virus.

Intraspecific transmission of Hantaan virus in the rodent Apodemus agrarius is as follows.

1) Apodemus mice were infected by intranasal route. 2) The large amounts of virus was found in saliva and urine but small amounts in feces. 3) Horizontal transmission of the virus among Apodemus mice occurred beginning 10 days after inoculation of the source cohort, and further groups of animals became infected when caged with Apodemus mice infected up to 38 days previously. Results were not different when ectoparasitized and clean animals were used in these experiments. 4) Attempts to demonstrate the virus in suspensions of ectoparasites harvested from infected Apodemus mice were failed.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animals, Resources, National Academy of Sciences-National Research Council.

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INTRODUCTION

Korean hemorrhagic fever (KHF) first attracted great attention during the Korean War when 2,422 U.S. soldiers were hospitalized with this disease (1). An ever-present health problem, approximately 300 to 900 persons are hospitalized with this disease every year, South Korea-wide (2).

Hemorrhagic fevers with a very similar syndrome to KHF have been reported as hemorrhagic fever with renal syndrome (HFRS) or hemorrhagic nephrosonephritis in Russia since 1913 (3.4) and several thousands cases occur annualy, as Songo fever or epidemic hemorrhagic fever (EHF) in China since 1931 (5.6.7) and more than 10,000 cases occur annualy, as nephropathia epidemica (NE) in Scandinavia since 1934 (8.9) and over 100 cases annualy, as epidemic nephritis or epidemic hemorrhagic fever in Eastern Europe since 1945 (10.11) and as epidemic hemorrhagic fever in Japan since 1960 (12).

The investigator et al reported the demonstration of a specific antigen and antibodies of KHF in 1976 (13) and discovered the etiologic agent and animal reservoir host of the disease and perfected a serologic test for diagnosis of the disease in 1978 (14). Very recently, the investigator et al. reported a close etiologic relation between KHF and HFRS (14), between KHF and NE, (15.16) and between KHF and EHF in Japan (17).

The etiologic agent of KHF was named as Hantaan virus after Hantaan river that runs along 38th paralell.

The physico-chemical properties of etiologic agent of KHF and mode of transmission of the agent in animal reservoir host are not known though mites had been suspected as the vector (3.7.18). The immunofluorescent technique (14) made possible to study some characteristics of the virus and an examination of the dynamics of transmission of the virus among Apodemus agrarius.

This report presents the results of the project on;

1) characterization of Hantaan virus and
2) mode of transmission of the virus in animal reservoir host, Apodemus agrarius coreae.

MATERIALS AND METHODS

Normal Apodemus agrarius for laboratory studies

Apodemus agrarius coreae were obtained at Chin Island and Apodemus agrarius jejuensis were trapped on Jeju Island. Neither island has ever registered cases of KHF. These animals weighed 20-50 g. Numerous graved animals were collected in spring and fall seasons and offspring of these were raised in the laboratory and used in some of the experiments.

Collection, identification and processing of rodents

Using live traps, rodents were captured in the field, in uncultivated scrub vegetation, and near farm dwellings.

Traps were set in the late afternoon and examined at midnight and at dawn. Animals were transported live to the laboratory in Seoul. Blood samples were obtained by cardiac puncture under chloroform anesthesia, and the animals were autopsied. Parotid glands, spleen, lungs, liver and kidneys were removed and weighed. Portions of each organ were titrated with BSS (pH 7.2) containing 1 percent bovine albumin (BSSA) for virus isolation, and the remainder was stored at -70° C.

Virus strains

Two virus strains were used in this work; Strain 76/118 was recovered from lung of a wild caught Apodemus agrarius rodent in 1976 and passaged 8 times in this species. The ID_{50} for Apodemus of the pool used was $10^{6.0}$/0.3 ml.
The 76/118 strain was used for characterization of the virus. The Lee strain was recovered from blood of a KHF patient and passaged 3 times in Apodemus and contained $10^{8.2}$ ID_{50}/0.3 ml. The Lee strain was used for transmission of the virus in Apodemus mice. Both strains were prepared as 10% w/v suspensions of lung tissue, and both were specifically neutralized by sera from a convalescent KHF patient and a rabbit infected with the KHF virus.

Detection of infection

Suspensions of tissues, secretions and ectoparasites were prepared in BSS, pH 7.2 containing 0.2% serum bovine albumin.

Fungizone and gentamicine in concentrations of 2 and 50 µg/ml were added to this diluent and suspensions were clarified by centrifugation at 1,000 g for 20 min. at 4° C prior to testing. Infectivity titrations were done by intramuscular inoculation of 3-4 Apodemus rodents per 10-fold dilution with 0.3 ml of suspension. Animals were sacrificed between 16-20 days later and frozen sections of lung tissue were examined by immuno-fluorescent as previously described (14). Specific antigen was taken as evidence of infection and titers were calculated according to Reed and Muench (19). In some cases tissues of experimentally infected animals were newly examined for KHF antigen and its presence was taken as evidence of viral infection. The indirect fluorescent technique employing FITC-conjugated anti-mouse immunoglobulin (Hyland laboratories, Costa Mesa, CA.) was used.

Experiments for characterization of the viurs

1) Filtration:

   Ten % suspension of infected Apodemus lungs were clarified by centrifugation at 1,000 g for 20 minutes at 4° C.
   The supernatant was filtered through both millipore and nucleopore filters of 0.05, 0.1, 0.22 and 0.45 micron pore size and the filtrates inoculated immediately into Apodemus.

2) Effect of BUDR on growth of the virus:

   A549 cells which support growth of Hantaan virus were used (20) to study effect of BUDR on growth of the virus.

Strain 76/118 after 7 passages in A549 cells was inoculated into A549 cells in maintenance medium containing 10^{-5} M/ml of BUDR (5'-Bromodeoxyuridine GIBCO, N. Y.) and growth of the virus was tested by FA technique. A549 cells in medium without BUDR were also used for controls.

3) pH stability:

Phosphate buffered saline (PBS), pH 7.2 was used to dilute the virus pools. One-unit of pH levels from 5.0 through 9.0 were achieved by the addition of varying amounts of phosphates. Specimens were incubated for 2 hours at 20-22° C in screw-cap tubes and shaked well at 10 minutes intervals. At the end of this period they were placed in an ice bath and infectivity was assayed immediately in Apodemus.

4) Thermal inactivation:

A virus pool suspended in BSS, pH 7.2, with 0.2% bovine albumin was used for thermal-inactivation studies. Two-ml volumes were dispensed into rubber-stoppered screw cap tubes. The tubes containing the virus aliquot were immersed in a water bath at 56° or 100° C, or placed in a 37° C incubator or on a 20° C laboratory bench or in a 4° C refrigerator, respectively, for designated intervals. Tubes were chilled in an ice bath and the contents immediately titrated in Apodemus.

5) Effect of diluent on infectivity of the virus:

Various diluents of pH 7.2, BSS or BSS with 0.2% bovine albumin or PBS or Tris buffered saline or veronal buffered saline, were mixed with a virus pool. Two-ml volumes were dispensed into rubber-stoppered screw cap tubes. The tubes containing the virus aliquot were placed on a laboratory desk, 20° - 22° C, for 2 hours shaking well at 10 minutes intervals. Tubes were chilled in an ice bath and the contents immediately titrated.

6) Lipid solvents and chemicals:

One-ml of virus suspension were mixed with 0.2 ml of chloroform or ether or BSS for controls, the mixtures were shaked well, incubated at 36° C for 10 min., and spined at 2,000 rpm for 7 min. at 4° C to separate the aqueous and solvent phase. Aliquots of virus suspension were mixed with equal volumes of SDC 0.1% final concentration, benzene, 0.5 N HCl, 0.05 N NaOH or 0.5% iodine solution or BSS for controls, and the mixtures were shaked well at 10 min. intervals for 60 minutes at 20° - 22° C. Then the mixtures were spined at 2,000 rpm for 5 min. at 4° C to separate the aqueous and solvent phases in those cases where two phases were separable. The aqueous phases of the mixtures were inoculated promptly into Apodemus for infectivity. Infected lung tissues were immersed into 100% acetone at 20° C for 7 min. and 10% suspension of

the lungs were made with the diluent and spined at 1,000 g for 20 min. at 4° C, and the supernatant was inoculated for infectivity. An aliquot of virus suspension was mixed with equal volume of 70% ethanol for 5 min. at 20° C and the mixture was inoculated promptly. Two parts of 20% suspension of infected lung tissues was mixed with one part of fluorocarbon and mixed at 15,000 rpm for 2 min. in Omnimixer. The mixture was centrifuged at 8,5000 rpm for 30 min. at 4° C and harvested the supernatant. The supernatant was inoculated promptly to Apodemus for infectivity. Lyphilization was done with virus pool made in BSS supplemented with 1% bovine albumin solution in Speedivac centrifugal freeze dryer, England. SDC was special enzyme grade (Mann Research Lab. N. Y.), diethyl ether was anhydrous and analytical grade (Mallenckrodt Chemical Works, St. Louis), absolute ethanol and acetone were reagent grade (Merck & Merck Co., U.S.), and NaOH, HCl and iodine were reagent grade (Wako pure chemicals industries, Japan). Fluorocarbon was commercial grade (Dupont chemicals, U.S.).

7) Effect of U.V. light:

Inactivation of the virus was made with strain 76/118 by irradiation of a 10^{-2} concentration of infected Apodemus lungs with ultraviolet light (Strato-ray, Air sanitizer); 1 ml of liquid in an uncovered Petri dish was exposed for 6 min. 3 inches from the lamp.

Before irradiation, the virus titer was $10^{-5.8}$ Apodemus ID_{50}/0.3 ml; after irradiation it was $10^{2.3}$/0.3 ml.

Experiments for virus transmission in Apodemus mice

1) Animal specimens:

Heparinized blood samples were obtained by cardiac puncture at intervals following experimental infection of Apodemus. Throat swab specimens were expressed into 1 ml of BSS to yield on estimated initial dilution of 1:50. Feces were collected during rodent autopsies to prevent urinary contamination and were homogenized in BSS to make 10% suspensions. Urine samples were collected as follows: Apodemus rodents, either individually or in groups of up to 10 which had been infected at the same time, were placed in specially designed funnel type metabolic cages fitted with 16 gauge stainless screens to prevent fecal contamination. Pears were used as food-water source and urine was collected during an interval of 3 - 5 hours, in sterile bottles containing 10 ml of BSS with 1% bovine serum albumin, pH 7.2. Fungizone and gentamicin in concentration of 2 and 50 µg/ml were added to this diluent. Before urine collection, funnel parts of cage was washed with 1% bovine albumin in BSS. Collection bottles were packed in wet ice. Urine pH was between pH 7.8 - 8.0 prior to assay for virus. Ticks, mites, fleas, and lice were brushed from Apodemus rodents, formed into pools of several hundred, and frozen at -60° C until

they were triturated and clarified to make 10% suspensions for virus assay.

2) Route of infection:

Susceptible Apodemus rodent were inoculated with 0.3 ml of serial virus dilutions by intramuscular, subcutaneous, intraperitoneal, intrapulmonary, oral and 0.1 ml by intranasal routes in order to determine differential sensitivity to infection.

3) Virus persistence:

Apodemus infected by intramuscular route with 1,000 ID_{50} of virus, Lee strain, were autopsied at intervals of up to one year in order to ascertain duration of virus infectivity and antigen persistence in various organs and secretions.

4) Virus transmission:

a) Groups of 10 Apodemus were inoculated intramuscularly with 1,000 ID_{50} of virus, Lee strain. Five uninoculated animals were placed in the same large cage with these animals on the day of inoculation. Every 5 days the exposed mice were removed from the infected cage and 5 new animals were introduced. Exposed animals were held in individual cages for a further period of 30 days. These were sacrificed and lung tissues were examined for presence of KHF antigen. The experiment was replicated except that both inoculated and exposed animals were first anesthetized, brushed and sprayed twice with

a 5% suspension of DDT in order to remove or kill all ectoparasites prior to inoculation with or potential exposure to virus.

b) Horizontal intercage transmission was tested by a series of trials in which 3 to 25 normal animals were kept for a certain period of time up to 5 months in cages kept 1 to 4 meters from and or the same height as a cage containing 3 to 12 inoculated Apodemus. Several exposed animals were then removed at 20 to 30 day interval and examined for KHF antigen in lung tissue. Three to 5 inoculated Apodemus in this and the proceeding transmission experiment were sacrificed 20 days after inoculation to be sure that the majority had become infected. KHF antigen was detected in at least 80% of all groups so examined. Sexual transmission of the virus was studied by exposing normal Apodemus mice to opposite sex mice infected with the virus.

c) Inability to establish a breeding laboratory colony of Apodemus agrarius severely limited attempts to determine whether the KHF agent could be transmitted vertically in this species. Several pregnant Apodemus captured in Chindo island were inoculated with 1,000 ID_{50} of viurs at Laboratory. Lung and other tissues from babies of these animals were also examined for presence of antigen.

RESULTS

A. No. of KHF patients in 1979

There were 364 hospitalized cases of KHF patients in South Korea in 1979 and 1 case was serologically diagnosed in US Army soldiers stationed in Korea, as shown in Table 1.

B. Physico-chemical properties of Hantaan virus

1) Size:

The virus passed through 100 nm Millipore filter although ID_{50} of the virus pool for Apodemus was dropped from $10^{6.8}$/0.3 ml. to $10^{3.2}$/0.3 ml. The virus did not pass 50 nm filter. In connection with findings of EM, size of the virus was estimated about 75 nm $\pm$ 5 nm with spherical shape. Observation of the virus particles under EM is in progress and it will be described in the next report.

2) Nucleic-acid determination:

Infectivity of Hantaan virus was unchanged when titrated in presence of BUDR. Titers of Hantaan virus and controls were $10^{6.3}$ and $10^{6.3}$/0.1 ml (Table 2.).

3) pH sensitivity:

Results are depicted in Figure 1. Hantaan virus infectivity titers were relatively stable between pH 7.0 and pH 9.0. Optimum pH for maximum infectivity were between pH 7.0 and pH 7.6. About one $\log_{10}$ reduction in titer occurred at pH 8.0 and above. The virus was almost completely inactivated at pH 5.0 and below.

4) Thermal stability:

Thermal-inactivation rates of Hantaan virus are shown in Figure 2. There was complete loss of detectable infectivity after 30 min. at 56° C and 48 hrs at 37° C. Although the virus was stable at 4° C, there was a 1,000-fold reduction in titer at the end of 28 days. There was detectable infectivity after 28 days at 20° C although titers were decreased from $10^{5.7}$ to $10^{1.3}$ ID_{50}/0.3 ml.

5) Effects of various diluents:

The effect of various diluent on infectivity of Hantaan virus is shown Fig. 3. The virus suspended in BSS and PBS for 2 hrs at 20° - 22° C had high titers of ID_{50} for Apodemus but the virus in Tris buffered and veronal buffered saline lost infectivity completely. The virus in BSS containing 0.2% serum bovine albumin was more stable than the virus in BSS. Therefore, virus pools used for study of infectivity were prepared in BSS, pH 7.2, containing 0.2% bovine albumin.

6) Effects of various chemicals on infectivity of Hantaan virus:

Virus suspension from Apodemus lung tissue was used. The lipid solvents (SDC, ether, chloroform, acetone, benzene, ethanol) and chemicals (HCl, NaOH, and iodine)

promptly and essentially completely inactivated Hantaan virus (Fig. 4).

C. Transmission of Hantaan virus in Apodemus agrarius

1) Susceptibility of Apodemus to KHF virus infection by different routes:

When Apodemus rodents were sacrificed 18-35 days after inoculation, it was found that all 6 routes employed resulted in successful infection. The intrapulmonary route gave the highest infectivity index and intranasal and oral routes were least efficient (Table 3) but these differences may be more apparent than real in view of the difficulty in obtaining complete administration of the inocula to the later two sites. As shown in Table 4, intranasal route of virus infection was as good as other routes. On the basis of these data, however, the intramuscular route was selected to infect rodents for transmission studies.

2) Persistence of virus, antigen and antibodies in infected Apodemus:

As a further prelude to transmission, serial observations were made on mice inoculated by intramuscular route. As shown in Table 5 and in Fig. 5. viremia was transitory from day 7 to 12 after inoculation. The pattern of virus distribution in kidneys and liver was not as diffuse as lungs and found tiny antigen spotts scarecely.

Virus was first detected in lungs 12 days after inoculation and persisted up to 350 days after infection (Fig. 5.). In lungs antigen but not infectious virus was found 160 to 365 days after infection. Antigen but not infectious virus was also demonstrated in parotid glands 46 to 260 days and in kidneys 43 to 60 days after infection. The presence of non-infectious virus antigen in these organs was shown as cross line in Figure 5. Salivary virus excretion was documented between 9 and 45 days post inoculation. Some specimens contained $10^{3.9}$ ID_{50}/ml. The large amounts of virus was found in urine, $10^{1.3}$ - $10^{4.1}$ ID_{50}/ml, between 9 to 106 days after infection. Virus was found in small amounts in feces between 12 and 45 days after infection. Amounts of virus in blood, excreta and various organs of Apodemus mice are shown in Table 3. Both neutralizing and immunofluorescent antibodies to virus were persisted for over 350 days post inoculation.

3) Horizontal transmission of KHF virus among Apodemus: As shown in Fig. 5, Apodemus mice exposed to infected Apodemus for 5 days in a same cage acquired infection beginning 10 days after inoculation of the source cohort, and further groups of animals became infected when caged with Apodemus infected up to 38 days previously. It is the period when virus was excreted in saliva, urine and feces. Results were not different when ectoparasitized

and clean animals were used in these experiments. No difference in transmission of the virus from infected male mice with virus to normal female mice and vice versa was observed as shown in Table 6. As shown in Table 7 baby mice were infected from infected mother. Horizontal intercage transmission of virus from infected mice in cages without protection covers to normal mice was demonstrated in a series of experiment as shown in Table 8 and it was necessary at least 40 days to became infected.

4) Attempts to demonstrate vertical transmission of Hantaan virus:

Attempts to demonstrate vertical transmission of virus from infected mother to fetus were failed in limitted experiments due to difficulties to breed mice in laboratory and in obtaining proper pregnant mice as shown in Table 9 and this experiment will be continued by inoculation of virus into pregnant mice which are collected in early stage of pregnancy during breeding season.

5) Attempts to demonstrate Hantaan virus in ectoparasites from infected Apodemus:

As shown in Table 10 attempts to demonstrate virus in mites, lice and fleas which were harvested from various stages of infected Apodemus mice were failed by inoculation of suspensions of ectoparasites into normal Apodemus mice.

DISCUSSION

Number of KHF patients among ROK Army soldiers after year of 1976 is decreased and this decrease is may be due to the results of eradication program of field rodents by ROK Army in 1976. ROK Army captured and killed about 120,000 field rodents in the south of the demilitarized zone, endemic areas of KHF, for prevention of KHF in summer and fall of 1976. Apodemus agrarius coreae, the animal reservoir host of KHF, is about 75% of total wild rodents in the endemic areas of the disease (21.22). We have named the etiologic agent of KHF as Hantaan virus after Hantaan river because i) Hantaan river runs along 38th Parallel and she is near Songnaeri where we have made the first isolation of the virus, ii) she runs through famous iron triangle areas where the first case of KHF and over 3,000 cases of the patients occurred in US troops during the Korean War, iii) Hantaan means lamentation in Korean and Hantaan river has many sad stories after 1945 because she is located between South and North Korea.

Physico-chemical properties of Hantaan virus indicated that it is a small cubic RNA virus, sensitive to lipid-solvents, heat-labile at above 37° C but stable relatively at below 20° C and sensitive to ordinary disinfectants. Hantaan virus does not have serologic relation with the etiologic agents of Marburg, Ebola and several arenaviruses (14). It may belong to a member of Bunyavirus or Orbivirus according to our limitted findings of EM studies which is in progress now.

The range of pH stability is similar to a wide variety of viruses and is of little help in classification, however, virus inactivation at pH 5.0 and below may afford a clue to explain the difficulty of oral infection of animals with acidic gastric secretions.

This is the first experiment in which dynamics of Hantaan virus transmission among Apodemus agrarius, the reservoir host for KHF, was investigated. Prior to this researchers had postulated that KHF was transmitted by ectoparasites harbored by various rats (3.7.18). Kitano et al. reported that they were able to induce KHF by inoculation of a suspension of ectoparasites from Apodemus agrarius into a monkey, but this finding has not yet been confirmed and this is the only positive report on vector of KHF so far published (7).

The key facts learned from these experiment are; 1) Viremia persists for 3 - 4 days in Apodemus agrarius. 2) Viral antigens can be isolated from the lung tissues of infected Apodemus agrarius for at least one year after viral inoculation, and some of these antigens exist in immune complexes. Viral antigens also may be found in the parotid glands, but in any case these antigens lose their infectivity after 160 days. 3) Large quantities of virus are excreted in the saliva and in the urine. 4) Regardless of the presence or absence of ectoparasites, the duration of infectivity of Apodemus agrarius is one month long

and coincides very nicely with the period during which the virus may be found in the saliva, urine and feces. 5) The main route of infection in Apodemus agrarius is through the respiratory tract, while infection via the G-I tract does not take place easily. It was thus demonstrated for the first time that ectoparasites are not the principle vector of KHF, as has long been theorized by many investigators.

We believe that infection can be transmitted via saliva or urine or feces of Apodemus agrarius to human and or other animals. There are large numbers of viral particles in saliva ($10^{3.9}$/ml), in urine ($10^{4.1}$/ml) and in feces ($10^{3.0}$/ml). Considering that we have demonstrated that ectoparasites are not needed for transmission of infection, we may conclude that no vector plays a significant role in the spread of disease.

When we look at the annual patient frequency chart, we note that there is small peak during the month of July and a larger peak during the months of October and November (2). It is entirely possible to explain these peaks on the basis of an excreta-borne transmission theory, as discussed below. 1) Many Apodemus agrarius which have been captured from endemic areas during the month of June, and during the months of September and October are found to be infected (14). There are two peaks of KHF patient in an year, small peak in July and large peak in October and November.

2) These two peak periods correspond with the dry season in Korea, when rain is infrequent and the air is dusty. June is the time for planting, while October through November is the time in which the farmers harvest their rice crops. It is probable that dust-borne, virus containing and sun-dried excreta are frequently aspirated by the farmer as he plows and digs the soil while planting and harvesting his crop.

3) During the harvest, there is naturally much grain in and around the villages, which in fact is often kept in places which are accessable to the rats such as Apodemus agrarius. The rats naturally are attracted to the villages during this period, thus coming into closer than usual contact with human.

4) There are two reproductive seasons for Apodemus agrarius; one during period of April-May-June and the other during the period of September-October-November. The animals copulate outside their burrows and their activities near villages increase in order to meet an increased demand for food during the period of pregnancy and subsequent birth of their offspring. Larger than usual amounts of excreta are found near the villages during these periods.

5) Apodemus agrarius are observed to clean themselves with their own saliva, and as do many other animals, they freely lick themselves and each other over their entire body, including their faces. It seems obvious that such animals, if infected, would by their nature tend to contaminate every place they go.

We have verified that rabbits, mice, rats, guinea pigs and monkeys go through a viremia stage after inoculation with KHF virus, and subsequently produce antibodies. Eventually, these animals may excrete the virus in their excreta. This implies that these animals may act as temporary carriers of the KHF agent and thus are a potential source of infection for human beings. Recently, rather conclusive evidence was forwarded that Hantaan virus was responsible for a series of outbreaks of KHF among the personels of experimental animal room of the Universities of Osaka, Wakayama, Niigata, Sendai, Kobe and Nagoya in Japan, and further that the disease was transmitted from colonized laboratory rats (23). Therefore, we strongly advocate that experiments with Hantaan virus in the animal room must be strictly regulated.

In colclusion, I would like to emphasize that Hantaan virus can be transmitted not only from striped field mice to other rodents, but also from these rodents to human and possibly from other animals to humans as well.

LITERATURE CITED

1. Smadel, J. E. Epidemic hemorrhagic fever. Amer. J. Pub. Health, 43:1327-1330, 1951.

2. Lee, H. W. Korean hemorrhagic fever, Ebola virus hemorrhagic fever. Else vier/North-Holland Biomedical Press, 331-342, 1978.

3. Smorodintsev, A. A., Dunaevskii, M. I., Kakhreidze, K. A., Neustroev, V. D. and Churilov, A. U. Etiology and clinics of hemorrhagic nephroso-nephritis. Moscow Medgiz, 26-47, 1944.

4. Smorodintsev, A. A., Kazbintsev, L. I. and Chumakov, V. G. Virus hemorrhagic fevers, Gimiz Gosndrastvennse Izdatel'stro Meditsinskoi Literatury, Leningrad, 1963, (Israel program for Scientific Translation. Jerusalem, 1964.) 1-245, Clearing House, Springfield, Va, 1964.

5. Ishii, S., Ando, K., Watanabe, N., Murakami, R., Nagayama, T. and Ishikawa, T. Studies on Songo fever. Japanese Army Med. J., 355: 1755-1758, 1942.

6. Kitano, M. A study of epidemic hemorrhagic fever. J. Manchou Med., 40: 191-209, 1944.

7. Kasahara, S., Kitano, M., Kikuchi, H., Sakuyama, M., Kanazawa, K., Nezu, N., Yoshimara, M. and Kudo, T. Studies on pathogen of epidemic hemorrhagic fever. J. Japanese Pathol., 34: 3-5, 1944.

8. Myhrman, G. Nephropathia epidemica, a new infectious disease in Northern Scandinavia. Acta Med. Scad., 140: 52-56, 1951.

9. Lähdevirta, J. Nephropathia epidemica in Finland. Ann. Clin. Res., 3: 12-17, 1971.

10. Gajdusek, D. C. Virus hemorrhagic fevers. J. Pediat., 60: 851-857, 1962.

11. Gaon, J., Karlovac, M., Gresikova, M., Hlaca, D., Rukavania, J., Kuezevic, V., Saratlic-savic, D. and Vampotic, A. Epidemiological features of hemorrhagic fever. Folia Medical Facultatis Medicinae Universitatis Saraeviensis, 3: 24-41, 1968.

12. Tamura, M. Occurrence of epidemic hemorrhagic fever in Osaka City: First cases found in Japan with characteristic feature of marked proteinuria. Biken J., 7: 79-94, 1964.

13. Lee, H. W. and Lee, P. W. Korean hemorrhagic fever. I. Demonstration of causative antigen and antibodies. Korean J. Internal Med., 19: 371-384, 1976.

14. Lee, H. W., Lee, P. W. and Johnson, K. M. Isolation of the etiologic agent of Korean hemorrhagic fever. J. Infect. Dis., 137: 298-308, 1978.

15. Lee, H. W., Lee, P. W. Lähdevirta, J. and Brummer-korvenkontio, M. Aetiological relation between Korean hemorrhagic fever and Nephropathia epidemica. Lancet, 1: 186-187, 1979.

16. Svedmyr, A. H., Lee, H. W., Berglund, A., Hoorn, B., Mystrom, K. and Gajdusek, D. C. Epidemic nephropathia in Scandinavia is related to Korean hemorrhagic fever. Lancet, 1: 109, 1979.

17. Lee, H. W., Lee, P. W., Tamura, M., Tamura, T. and Okuno, Y. Etiological relation between Korean hemorrhagic fever and epidemic hemorrhagic fever in Japan. Biken J., 22: 1-4, 1979.

18. Traub, R., Marshall, H., Lawrence, W. H. and Harris, T. T. Potential vectors and reservoirs of hemorrhagic fever in Korea. Am. J. Hyg. 59: 291-305, 1954.

19. Reed, L. J. and Muench, H. V. A simple method of estimating fifty percent end points. Am. J. Hyg., 27: 493, 1938.

20. French, G. R., Foulke, R. S., Brand, O. A., Eddy, G. A., Lee, H. W. and Lee, P. W. Propagation of etiologic agent of Korean hemorrhagic fever in a cultured continous cell line of human origin. To be published.

21. Kim, I. S., Lim, Y. W. and Lee, H. W. Studies on field rodents collected in the hyperendemic areas of KHF. J. Korea Med. Univ., 11: 263-291, 1974.

22. Kwon, C. S,., Lim, Y. W. and Lee, H. W. Studies on ectoparasites of field rodents collected in the endemic areas of Korean hemorrhagic fever. J. Korea Med. Univ. 10: 817-827, 1983.

23. Umenai, T., Lee, H. W., Lee, P. W., Saito, T., Toyoda, T., Hongo, M., Yoshinaga, K., Nobunaga, T. Horiuchi, T. and Ishida, N. Korean hemorrhagic fever in staff in an animal laboratory. Lancet, 1914-1316, 1979.

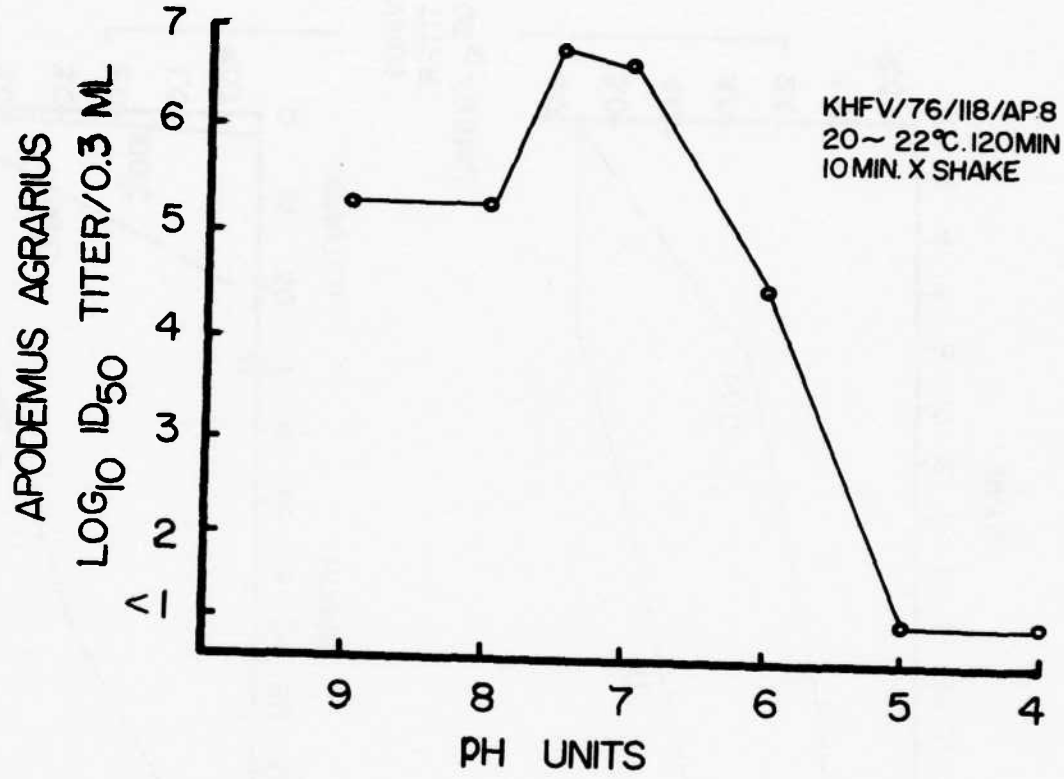

FIG. 1. THE EFFECT OF PH OF PBS ON THE INFECTIVITY OF KHF VIRUS

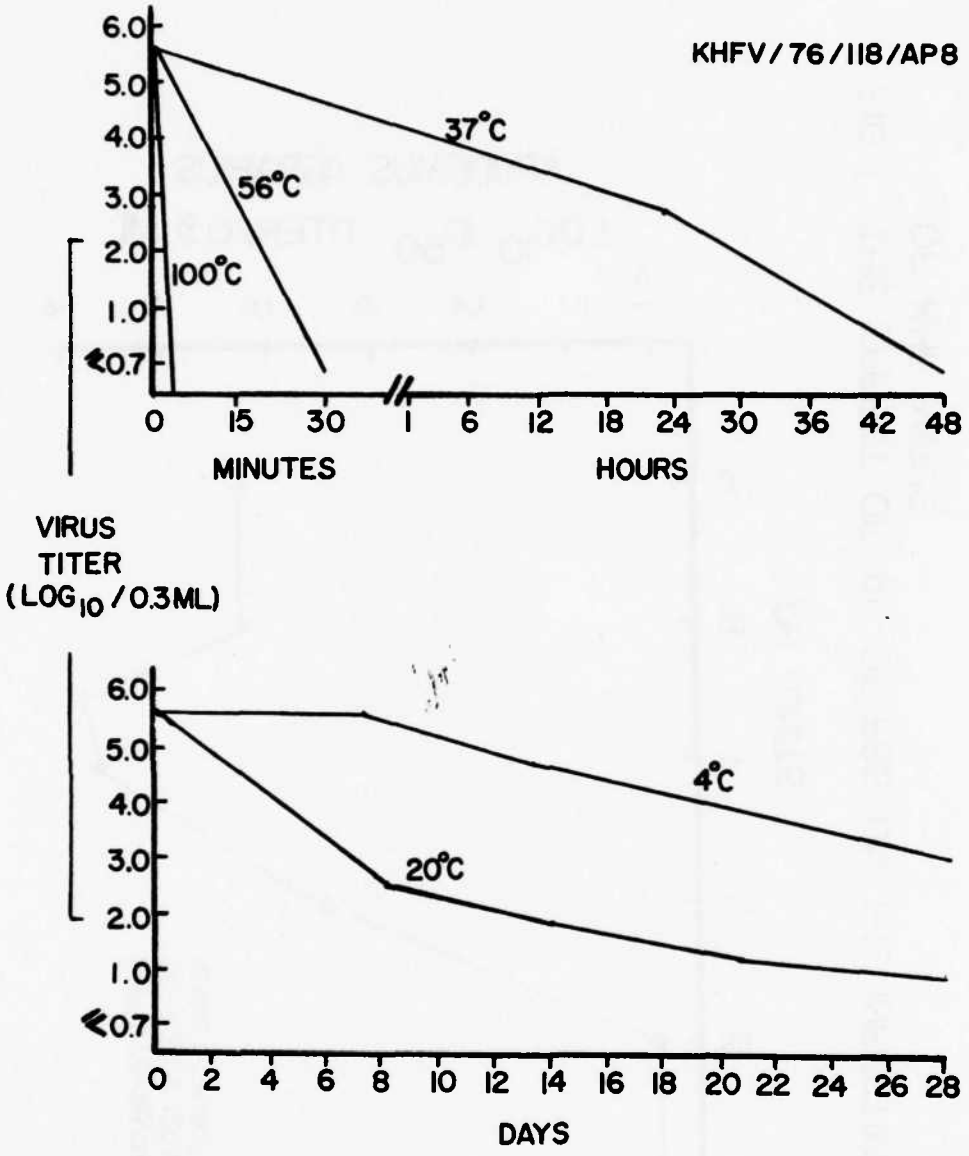

FIG. 2. THERMAL-INACTIVATION RATES OF KHF VIRUS

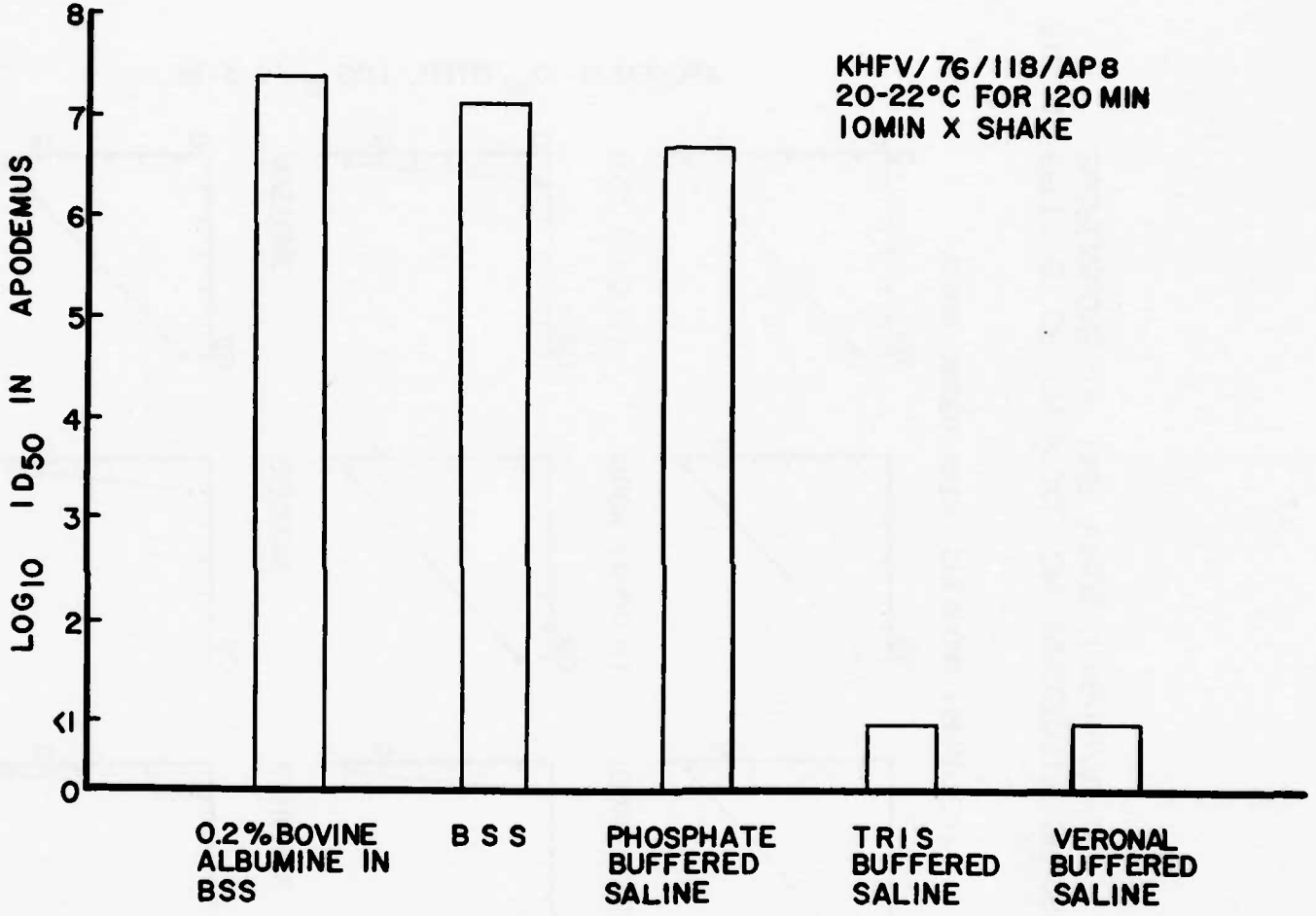

FIG. 3. THE EFFECT OF DILUENTS (pH 7.2) ON THE INFECTIVITY OF KHF VIRUS

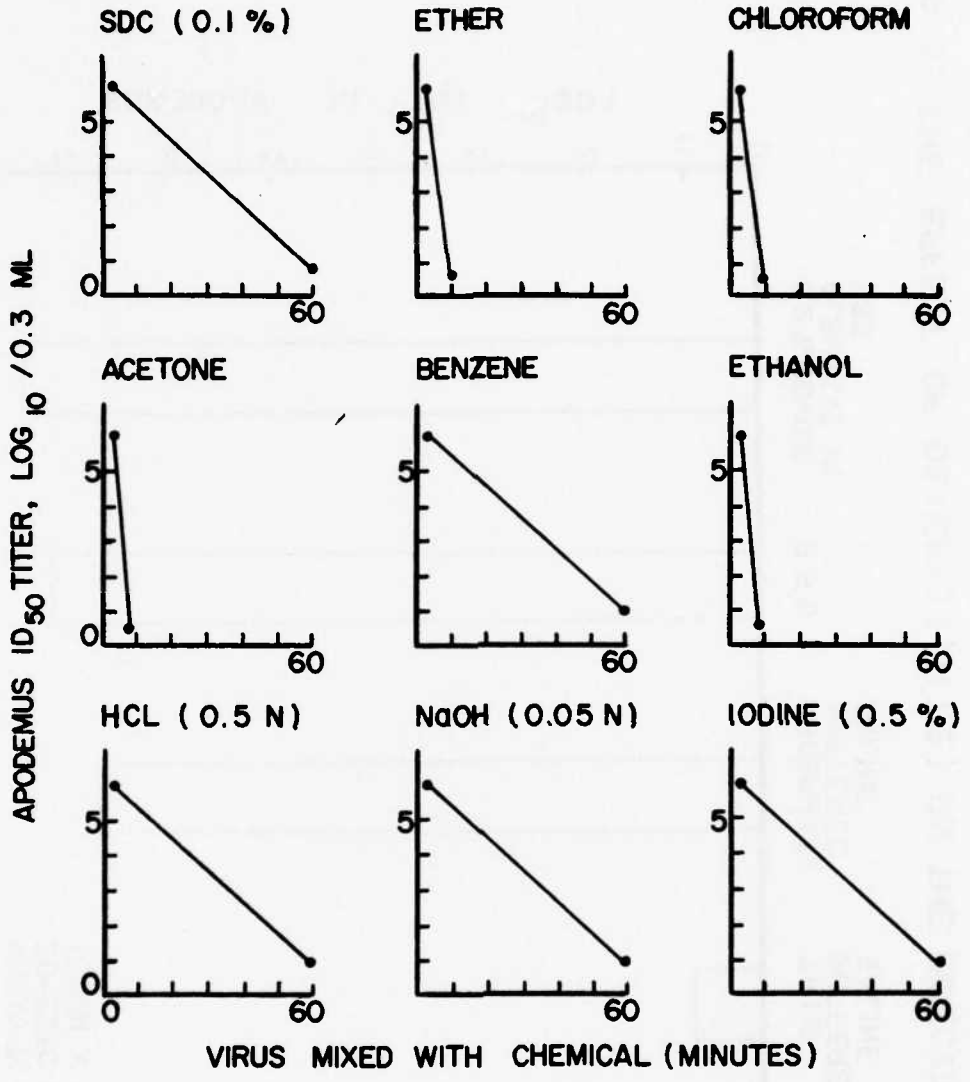

FIG. 4. EFFECTS OF CHEMICAL ON INFECTIVITY OF CRUDE SUSPENSIONS OF KHF VIRUS (76/118/AP8)

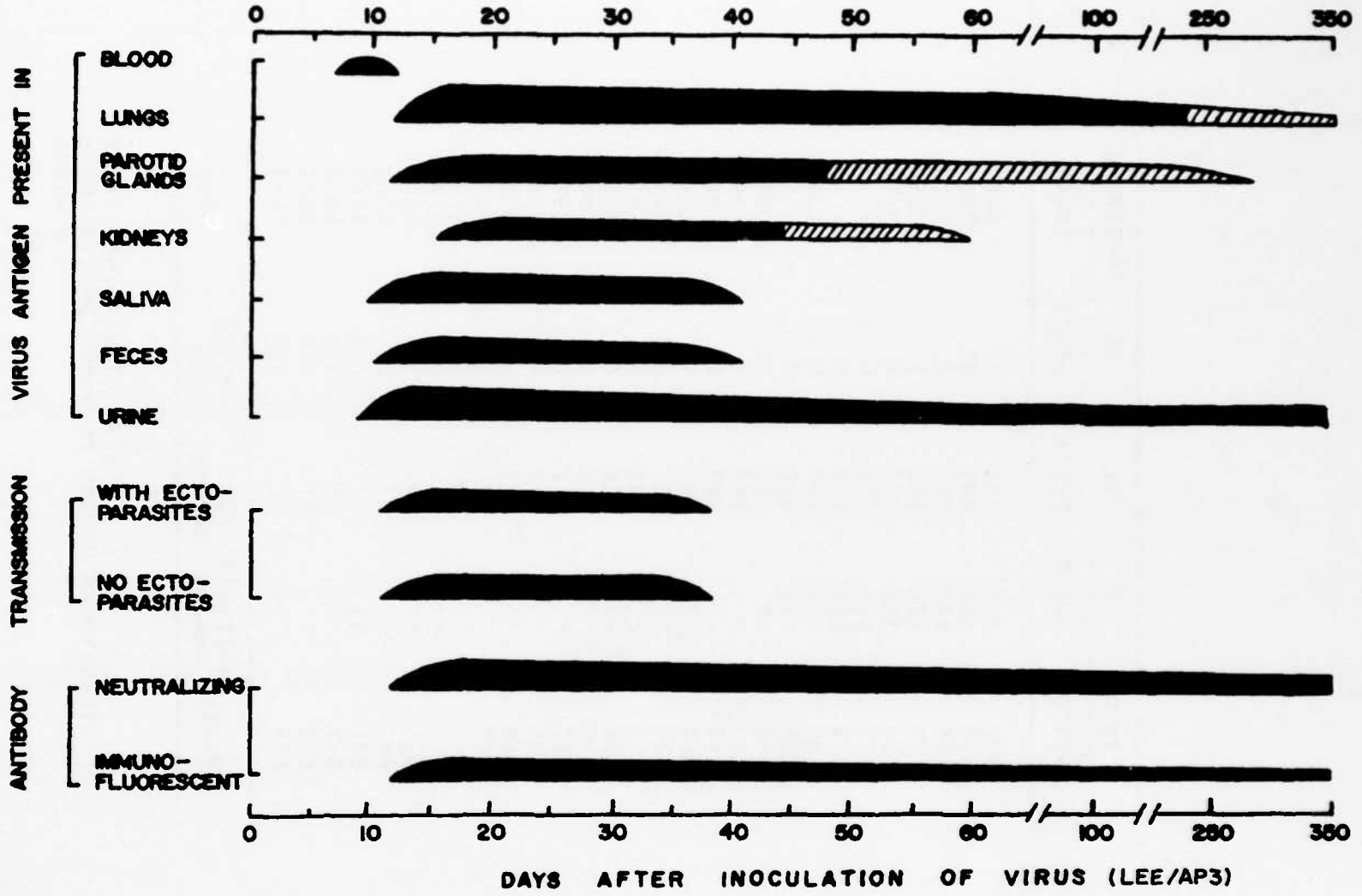

FIG. 5.
THE COURSE OF INFECTION AND INFECTIVITY OF HANTAAN VIRUS IN APODEMUS AGRARIUS

Table 1
Hospitalized cases of Korean hemorrhagic fever patients in 1979

Year	US forces	Korean army	Korean civilian	Total
1951	827	...	...	827
1952	833	...	...	833
1953	455	...	...	455
1954	307	...	19	326
1955	20	...	...	20
1956	28	26	...	54
1957	13	21	...	34
1958	15	20	...	35
1959	79	47	...	126
1960	10	185	...	195
1961	27	341	...	368
1962	29	311	...	340
1963	11	257	...	268
1964	22	205	18	245
1965	99	110	2	211
1966	36	82	11	129
1967	31	86	13	130
1968	28	102	13	143
1969	9	134	8	151
1970	13	221	85	319
1971	2	358	311	671
1972	0	203	186	389
1973	0	237	241	478
1974	0	251	170	421
1975	1	370	466	837
1976	4	304	521	829
1977	7	212	288	507
1978	10	168	201	379
1979	1	122	241	364
Total	2,917	4,373	2,794	10,084
Fatality	5%	7%	8%	6.6%

Table 2
Physico-chemical properties of Hantaan virus, etiolotic agent of KHF

Description	Properties
Size	@ 75 nm
Morphology	Spherical
Filtration 100 nm	Passed
50 nm	Not passed
BUDR	Resistant
Deoxycholate (0.1%)	Inactivated
Ether	Inactivated
Chloroform	Inactivated
Acetone	Inactivated
Benzene	Inactivated
Fluorocarbon	Inactivated
Ultraviolet light	Inactivated
Lyophilization	Stable
pH 5	Inactivated
pH 9	Stable
Heat 56° C 30 min.	Inactivated
100° C 1 min.	Inactivated
0.5 N HCl	Inactivated
0.05 N NaOH	Inactivated
0.5% iodine	Inactivated
70% ethanol	Inactivated

Table 3
Comparative infectivity of Hantaan virus in Apodemus agrarius by different route of infection

Virus dilution	Route of inoculation					
	IM/0.3	SC/0.3	IL/0.1	IP/0.3	IN*/0.1	Oral*/0.3(ml)
10^{-2}					4/5	1/2
10^{-3}	3/3	3/3	3/3	3/3	3/5	4/5
10^{-4}	5/5	3/3	3/3	3/3	2/5	0/3
10^{-5}	6/6	2/3	2/3	1/3	0/3	0/5
10^{-6}	2/6	0/3	3/3	0/3	0/4	0/5
10^{-7}	1/6	0/3	1/3	0/3		
ID_{50}/0.3 ml	$10^{-5.9}$	$10^{-5.2}$	$10^{-7.0}$	$10^{-4.7}$	$10^{-3.8}$	$10^{-3.2}$

* Animals were killed on 35 days after inoculation

Inoculated virus was 76/118 AP8 and animals were autopsied on 18 days after inoculation. Lungs are excised and examined by FAT.

Table 4
Infection of Hantaan virus to Apodemus agrarius by intranasal route (118/AP8 1,000 ID_{50}/0.1 ml)

	Presence of virus on days after inoculation						
	7	10	15	20	25	30	40
Blood	3/3[1]	3/3	0/3				
Saliva	1/3	3/3	3/3	3/3	2/3	3/3	2/3
Urine	4/4	3/3	3/3	3/3	3/3	2/3	3/3
Feces			0/3	3/3	2/3	0/3	1/3
Lungs			0/2	3/3	3/3	3/3	2/3
Parotid glands			0/2	3/3	3/3	2/3	2/3

1 No. positive/No. inoculated

Table 5
Amount of Hantaan virus in the excreta and organs of Apodemus agrarius coreae infected with 1,000 ID_{50} of virus (Lee strain AP3)

Excreta & organs	Apodemus -log ID_{50}/ml or g. on days after virus inoculation									
	7	9	12	15	25	35	45	86	141	158
Blood	1.0	2.4	1.9	≤0.5						
Saliva		≤2.1	2.6	3.9	3.6	2.2	≤2.1			
Urine		1.8	3.8	3.8	4.1	2.2	1.8	2.8	2.3	1.8
Feces		≤1.5	2.5	3.3	3.0	1.8	≤1.5			
Lungs			n.t.	8.7	n.t.					
Kidneys			n.t.	7.2	n.t.					
Liver			n.t.	6.7	n.t.					
Submaxillary & parotid glands			n.t.	8.2	n.t.					
Lacrimal glands			n.t.	5.6	n.t.					

n.t. : not tested

Table 6
Horizontal transmission of Hantaan virus from infected Apodemus agrarius to normal Apodemus agrarius in a cage

Sex & no. of inoculated Apodemus with virus[1]	Sex & no. of normal Apodemus mixed to infected Apodemus	Duration of co-living in a cage	No. positive/no. tested	
			Inoculated Apodemus with virus	Normal Apodemus
Male 4	Male 5	32 days	3/4	4/5
Male 5	Female 4	32 days	4/5	4/4
Female 5	Male 5	32 days	4/5	4/5
Female 5	Female 5	32 days	3/5	5/5

1 Inoculated Apodemus mice were on 10 days after inoculation of 1,000 ID_{50} virus, Lee AP3, when mixed with normal Apodemus mice.

Table 7
Horizontal transmission of Hantaan virus from infected mother Apodemus agrarius to her baby mice

Code of pregnant mouse	Dose & route of virus inoculation into pregnant	Examined lung of mother for presence of virus on day after virus inoculation		No. of baby born on day after virus inoculation	Examined baby on 46 days after virus inoculation into mother, No.infected/ no.baby tested
PAP-1	76/118 AP8 1,000 ID_{50}/I.M.	++++[1]	day 15	4 mice on day 1	1/4
PAP-2	"	+++	day 15	5 mice on day 1	2/5
PAP-3	"	++++	day 15	4 mice on day 3	3/4
PAP-4	Lee/AP3 1,000 ID_{50}/I.M.	+++	day 46	4 mice on day 5	1/4

1 Presence of Hantaan virus antigen in lung was indicated by + to ++++.

Table 8
Horizontal intercage transmission of Hantaan virus from infected Apodemus agrarius to normal Apodemus agrarius

Experiment No.	No. of cages with infected Apodemus (no. of mice)	Distance between cages with infected and normal mice, meter	No. of normal Apodemus in a cage	Examined normal Apodemus on days after	No. infected / No. tested
1	15 (45 mice)	4	11	90 105	3/4 6/7
2	20 (60 mice)	1	3	120	2/3
3	2 (25 mice)	2	25	30 60 90 120 150	0/5 0/5 0/5 5/5 5/5
4	1 (12 mice)	2	16	20 40 60 80	0/4 1/4 3/4 4/4

Table 9
Attempt to demonstrate vertical transmission of Hantaan virus in pregnant Apodemus agrarius

Code of pregnant mice	Strain, dose and route of inoculation	No.of baby born on days after virus inoculation into mother	Killed baby mice on day after birth	No.infected/ no.baby tested	Virus antigen in lung of mother when killed with sucklings
P79AP-1	76/118 AP8 1,000 ID_{50} I.M.	3 mice on day 3	14	0/3	+++
P79AP-2	"	4 mice on day 6	11	0/4	+++
P79AP-3	"	4 mice on day 7	20	0/4	+
P79AP-4	"	4 mice on day 7	20	0/4	+

Table 10 Attempts to isolate Hantaan virus from suspension of ectoparasite harvested from infected Apodemus agrarius with Hantaan virus

Ectoparasite Name	No.	Time of collection from infected Apodemus with Hantaan virus	No. infected/ no. inoculated[1]
Mites[2]	4,000	Harvested from Apodemus collected in endemic area from May.- Aug. 1977.	0/3
Mites	4,000	Harvested from Apodemus collected in endemic area from Sep.- Dec. 1977.	0/3
Mites	5,000	Harvested from Apodemus collected in endemic area from Mar.- Jun. 1978.	0/3
Mites	850	Harvested from Apodemus collected in endemic area from Oct.- Nov. 1978.	0/3
Mites	4,500	Harvested from infected Apodemus on 16-20 days after inoc. of virus.	0/5
Mites	4,400	Harvested from infected Apodemus on 16-60 days after inoc. of virus.	0/6
Mites	2,600	Harvested from infected Apodemus on 20-200 days after inoc. of virus.	0/3
Lice[3]	1,500	Harvested from Apodemus collected in endemic area from May.- Aug. 1977.	0/3
Lice	1,500	Harvested from Apodemus collected in endemic area from Sep.- Dec. 1977.	0/3
Lice	1,500	Harvested from Apodemus collected in endemic area from Mar.- Jun. 1978.	0/3
Lice	320	Harvested from Apodemus collected in endemic area from Oct.- Nov. 1978.	0/3
Fleas[4]	85	Harvested from Apodemus collected in endemic area from Sep.- Dec. 1977.	0/3

1 Apodemus was autopsied on 20th day after inoculation of 0.5 ml of the suspension intramuscularly for detection of virus infection.
2 Laelaps agilis 40%, Leptatrombidium pallida 17%, Lept. palpalis 16% and others.
3 Polyplax serrata 50%, Polyp. spinulosa 25% Halpo. oenomydis 15% and others.
4 Ctenophthalmus congener 68%, Xenopsylla cheopis 13% and others.

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LIST OF PUBLICATIONS

1. Lee, H. W., Lee, P. W. Lähdevirta, J. and Brummer-korvenkontio, M. Aetiological relation between Korean hemorrhagic fever and Nephropathia epidemica. Lancet, 1: 186-187, 1979.

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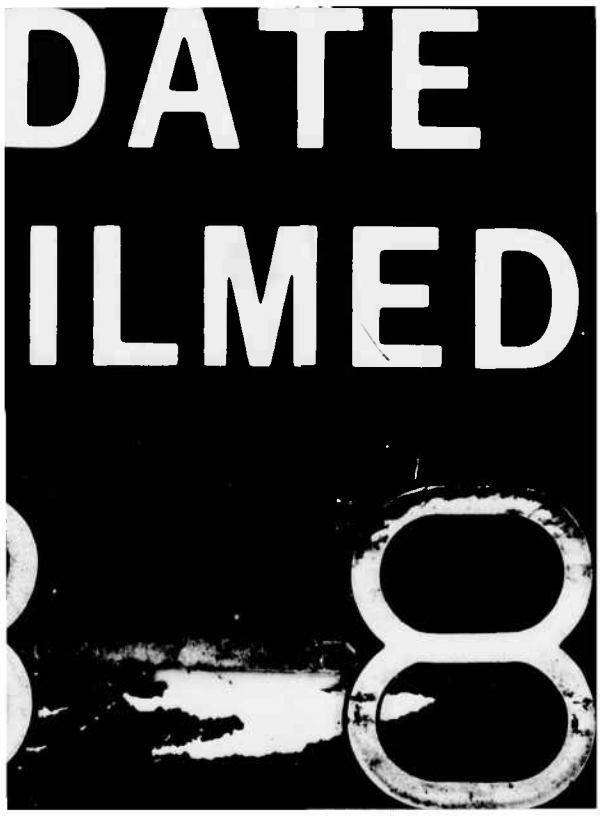
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